Invited Review

Grain dormancy genes responsible for preventing pre-harvest sprouting in barley and wheat

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Pre-harvest sprouting (PHS) remains a long-standing problem for the production of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) worldwide. Grain dormancy, a key trait for the prevention of PHS, controls the timing of germination. Discovery of the causal sequence polymorphisms (CSPs) that produce naturally occurring variation in dormancy will help improve PHS tolerance. The identification of CSPs for dormancy remains difficult, especially for barley and wheat, because they are the last major cereals to have their genomes sequenced. However, recent work has identified several important CSPs that play pivotal roles in fine-tuning the dormancy levels in barley and wheat cultivars. This review summarizes these recent advances, which can be directly applied in breeding programs to improve PHS tolerance. These recent findings indicate the possibility that barley and wheat cultivars grown in East Asia, where much rain falls during the harvest season, will be rich sources of alleles that confer strong dormancy, since these cultivars have been selected to cope with the regional climate. The newly discovered dormant alleles will be useful for improving PHS tolerance around the world, just as *Reduced-height (Rht)* alleles from Japanese wheat varieties contributed to yield increases for the Green Revolution.

Key Words: barley, wheat, pre-harvest sprouting, seed dormancy, QTL.

Introduction

Pre-harvest sprouting (PHS), a phenomenon where seeds germinate on the plant before harvest, is a major problem around the world, especially for barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), the fourth and second most widely produced cereals. PHS causes devastating damage to barley and wheat production because yields are reduced and the resulting grain is of lower quality, leading to major economic losses.

Barley and wheat originated in the Fertile Crescent (Kilian *et al.* 2009), whose Mediterranean climate is characterized by hot, dry summers and cool, moist winters. In this region, little rain falls during the harvest season (usually around June), so PHS is rare. However, since the end of the last major glacial period about 10,000 years ago, the distribution of barley and wheat cultivars has expanded across the world and today, barley and wheat are cultivated in more than 100 countries. In some parts of these countries, these grains are cultivated in climates where rain tends to fall dur-

ing the harvest season, and PHS damage has been reported, including Japan and the major wheat-producing countries of China, India, the USA, Canada, Germany, and Australia (Derera 1990, Liu *et al.* 2016, J.-R. Wang, personal communication 2016). Therefore, PHS remains a problem that must be solved to prevent economic losses and improve food security. Breeding programs have improved PHS tolerance in barley and wheat varieties, and the resulting varieties are much more tolerant to PHS than older ones, but the level of tolerance is not yet sufficient to prevent PHS completely (Fig. 1).

A key trait for the prevention of PHS is grain dormancy, which is the suppression of the germination of grains (or seeds), even under favorable (wet) conditions (Finkelstein *et al.* 2008, Nonogaki 2014, Rodríguez *et al.* 2015). Dormancy is a quantitative trait regulated by multiple genes, and is strongly influenced by environmental conditions. From an agricultural perspective, it is desirable for cultivars to have grains that remain dormant until harvest to prevent PHS; however, the dormancy should quickly disappear after harvest to allow the grains to germinate rapidly and almost simultaneously at sowing (or, in the case of malting barley, at malt production). To select genotypes with this ideal pattern of dormancy, it is necessary to understand the genetic regulatory mechanisms underlying this phenomenon. This

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Fig. 1. Pre-harvest sprouting (PHS) barley and wheat varieties. In pre-harvest sprouting (PHS), grains germinate on the spike after rain during the harvest season. The images show PHS in barley varieties (A: two-row malting barley; B: six-row barley) grown in Tochigi, Japan, in 2014, and PHS in wheat varieties (C and D) grown in Hokkaido, Japan, in 2016. In A, only white roots emerged from grains. In B, C, and D, green shoots emerged from grains.

review summarizes recent advances in the understanding of quantitative trait loci (QTL) for grain dormancy in barley and wheat.

Analysis of QTL for grain dormancy in barley and wheat: towards cloning the causal genes

QTL analysis is a powerful tool in the genetic analysis of quantitative traits (Tanksley 1993). The first reports of a QTL for grain dormancy in barley and wheat were published in the early 1990s (Anderson *et al.* 1993, Ullrich *et al.* 1992). Since then, dormancy QTL have been found on all chromosomes in both barley and wheat (Flintham *et al.* 2002, Kulwal *et al.* 2010, Mares and Mrva 2014, Nakamura *et al.* 2017). Interestingly, among them, only a few dormancy QTL have large effects. Barley has two major dormancy QTL, *SD1* and *SD2*, and wheat has several dormancy QTL, including *QPhs.ocs-3A.1* and *Phs1* (Gong *et al.* 2014, Mori *et al.* 2005, Torada *et al.* 2005, 2008).

Genetic markers for these major QTL would be valuable for breeders because the QTL are likely to express their effects under different environmental conditions (Tanksley 1993). Therefore, it is important to identify the causal sequence polymorphisms (CSPs) for these major QTL to develop markers for selection of the QTL. For such analyses, fine mapping of the genes with precisely annotated genomic sequences is indispensable. However, compared with model plants such as *Arabidopsis thaliana* and rice (*Oryza sativa*), and even with other major crops such as corn (*Zea mays*) or soybean (*Glycine max*) (IRGSP 2005, Schmutz *et al.* 2010, Schnable *et al.* 2009, The *Arabidopsis* Genome Initiative 2000), it was much more difficult to decode the barley and wheat genome sequences, because they are much larger (approximately 5 Gb and 17 Gb, respectively) and contain a lot of repeated sequences. In addition, wheat is allohexaploid, making the determination of its genome sequence even more challenging. Although the barley reference genome sequence was published recently (Mascher *et al.* 2017) and the wheat reference genome sequence v.1.0 is available in 2017 from the International Wheat Genome Sequencing Consortium (<https://www.wheatgenome.org/>), and will likely be published soon (Callaway 2017), the lack of a reference genomic sequence for barley or wheat had made it much more difficult to identify the causal genes for QTL responsible for dormancy, even those with large effects. Moreover, due to the complex nature of seed dormancy, to date, even in model plants, only a few causal genes for dormancy QTL have been identified, e.g. *DELAY OF GERMINATION 1 (DOG1)* in *Arabidopsis*, and *Sdr4* in rice (Bentsink *et al.* 2006, Sugimoto *et al.* 2010). However, dormancy is so important for improving PHS tolerance in barley and wheat, it is necessary to identify the natural allelic variants that affect dormancy, so that these alleles can be used in barley and wheat breeding programs.

Around the year 2000, several research groups around the world began trying to identify causal genes for the major dormancy QTL of barley and wheat. After more than a decade of hard work, these studies eventually revealed that *Alanine aminotransferase (AlaAT)* is the causal gene for the major grain dormancy QTL *Qsd1 (SD1)* in barley (Sato *et al.* 2016), and that *Mitogen-activated Protein Kinase Kinase 3 (MKK3)* is the causal gene for the major grain dormancy QTL *Qsd2-AK (SD2)* in barley and *Phs1* in wheat (Nakamura *et al.* 2016, Torada *et al.* 2016). Moreover, in the course of these studies, *Mother of FT and TFL1 (MFT)* was found as the causal gene for the major wheat grain dormancy QTL *QPhs.ocs-3A.1* (Nakamura *et al.* 2011).

AlaAT is the causal gene for the major dormancy QTL *Qsd1* in barley

In barley, *Qsd1* was identified as a single major dormancy QTL using doubled haploid lines derived from ‘Haruna Nijo’ and the wild barley accession ‘H602’ (Hori *et al.* 2007). Haruna Nijo is a malting barley, and has very weak

Table 1. Major QTL for grain dormancy in barley and wheat

	Diploid barley		Hexaploid wheat	
Dormancy QTL	<i>Qsd1</i>	<i>Qsd2-AK</i>	<i>QPhs.ocs-3A.1</i>	<i>Phs1</i>
Chromosome	5HC	5HL	3AS	4AL
Causal gene	<i>AlaAT</i>	<i>MKK3</i>	<i>MFT</i>	<i>MKK3</i>
Tissue expression	Embryo	All organs (low levels)	Embryo Coleorhiza	All organs (low levels)
CSP	G642C	A779C	T(-222) C	C660A
Mutation site	Coding sequence (L214F)	Coding sequence (N260T)	Promoter sequence (A-motif)	Coding sequence (N220K)
Mutation function	ND	Reducing kinase activity	Maintaining expression	ND

A-motif, a 6-bp palindromic sequence (TACGTA); C, centromere; CSP, causal sequence polymorphism (wild type to mutant); L, long arm; Low levels, gene expression levels are low and stable; ND, not determined; QTL, quantitative trait locus/loci; S, short arm.

dormancy, with germination rates that are always close to 100%. By contrast, H602 is a wild progenitor of cultivated barley (*H. vulgare* ssp. *spontaneum*), and has very strong dormancy with a germination rates that are always close to 0% at harvest. As a single major QTL, *Qsd1* has an extremely strong effect on dormancy, explaining more than 50% of the phenotypic variation in dormancy levels between these two parental lines (Hori *et al.* 2007).

AlaAT was identified as the causal gene for the *Qsd1* QTL using map-based cloning (Sato *et al.* 2016). *AlaAT* (EC 2.6.1.2), also known as ALT, interconverts the amino acid residues glutamate and alanine by transferring an amino group from alanine to oxaloacetate to form pyruvate and glutamate (Welch 1972). Mammalian studies have revealed that *AlaAT* is crucial in gluconeogenesis and amino acid metabolism and this familiar enzyme is used as a marker of liver function (Felig 1973). Almost all living organisms have *AlaAT*, including Archaea, implying that it is ancient and highly conserved (Sakuraba *et al.* 2004). In plants, *AlaAT* is involved in nitrogen assimilation, protein synthesis, and carbon metabolism (Duff *et al.* 2012). However, the mechanism underlying the role of *AlaAT* in the regulation of dormancy remains unknown. One hypothesis is that *AlaAT* might alter grain color, which has been suggested to affect dormancy (Debeaujon *et al.* 2000, Flinham 2000, Himi and Noda 2005). In barley, proanthocyanidin accumulation in the testa of developing grains was reported to affect grain dormancy (Himi *et al.* 2012). Chalcone is an intermediate metabolite made during the synthesis of proanthocyanidin. Free alanine residues in the embryo are used to make chalcone for pigmentation synthesis in the seed coat of *Brassica napus* (Wang *et al.* 2016). Thus, different *AlaAT* genotypes might affect dormancy through regulation of grain color; however, so far, there is no evidence to suggest that *AlaAT* is involved in the regulation of chalcone biosynthesis in barley.

Association analysis between *AlaAT* genotypes and dormancy phenotypes has suggested that a single nucleotide polymorphism (SNP) G642C at exon 9 of *AlaAT* could be a CSP for dormancy. This SNP causes substitution of the 214th amino acid residue from leucine (L) in the dormant allele to phenylalanine (F) in the non-dormant allele in *AlaAT* (Table 1), though the effect of this substitution on the function of *AlaAT* remains unknown. The L214F substi-

Fig. 2. Dominance and relative effects of mutant alleles on dormancy. The relative levels of dormancy caused by each mutation are compared with those of the wild-type allele. The starting and the ending points (heads) of the arrows show the relative dormancy levels of the wild-type allele and the mutant allele of each gene, respectively. The arrow's length represents the qualitative effect of each QTL. Blue arrows show increasing dormancy levels produced by the mutations. Green arrows show decreasing dormancy levels produced by the mutations. Red and black letters of the QTL and gene names indicate the mutations were estimated to have originated in East Asia and West Asia, respectively.

tution site is not close to the active site, the cofactor binding site, or the junction zone of the two polypeptides of the dimer (Sato *et al.* 2016). It has been speculated that the region is more likely to be involved in dimer formation, or in binding another regulatory protein. The F214 non-dormant mutation allele is dominant (Fig. 2) (Sato *et al.* 2009, 2016). This mutation is thought to have occurred in West Asia soon after domestication, resulting in the transition from strong to weak dormancy in cultivated barley (Sato *et al.* 2016).

***MKK3* is the causal gene for the major dormancy QTL *Qsd2-AK* in barley**

Using recombinant inbred lines (RILs) from the old Japanese varieties 'Azumamugi (Az)' and 'Kanto Nakate Gold' (KNG), a single major dormancy QTL, *Qsd2-AK*, was found at the *SD2* locus. Az, which was bred from Japanese

landraces (Sameri *et al.* 2006), is a six-row barley variety that is used for food and has strong dormancy. KNG is a two-row malting barley with weak dormancy and is derived from a malting variety ‘Golden Melon’, which was imported from northern Europe to Japan in 1881 (Sameri *et al.* 2006, Takahashi 1980). Recently, using map-based cloning, the causal gene for the QTL *Qsd2-AK* was identified as *MKK3* (Nakamura *et al.* 2016).

MKK3 functions in the well-known, conserved mitogen-activated protein (MAP) kinase cascade signal transduction pathway. In general, a MAP kinase cascade consists of three modules: a MAP kinase kinase kinase (MAP3K), a MAP kinase kinase (MAP2K), and a MAP kinase (MAPK). In response to external signals, the MAP kinase cascade successively transmits the signals via phosphorylation to downstream factors, such as transcription factors, to control cell growth and proliferation. Plants have many more of these modules than animals; being sessile, plants must respond appropriately to environmental signals to survive. Plants such as *Arabidopsis* and rice have about 80 MAP3Ks, 10 MAP2Ks, and 20 MAPKs (Ichimura *et al.* 2002). MAP3K signals converge on the intermediate module MAP2Ks; thus MAP2Ks play pivotal roles in the MAP kinase cascade. *MKK3* is a member of the MAP2K family with a unique structure, having a kinase domain and nuclear transport factor 2 (NTF2) domain together in the same MAP2K. *MKK3* is present in all photosynthetic eukaryotes, which means its origin can be traced back at least 800 million years to when single-celled algae first emerged (Ramanan *et al.* 2016). *MKK3* has been reported to function in several signaling pathways such as those involved in pathogen resistance (Dóczi *et al.* 2007), jasmonic acid signaling (Takahashi *et al.* 2007), wounding signals (Takahashi *et al.* 2011), osmotic stress and abscisic acid (ABA) signaling (Zhang *et al.* 2012), and blue light signaling (Sethi *et al.* 2014). Recent *Arabidopsis* studies have revealed that *MKK3* functions in an ABA-activated MAPK cascade comprising the MAP3Ks MAP3K17 and MAP3K18, the MAP2K *MKK3*, and the MAPKs MPK1, 2, 3, 7, and 14 (Colcombet *et al.* 2016, Danquah *et al.* 2015, Matsuoka *et al.* 2015). This cascade is involved in the ABA stress signaling pathway. The plant hormone ABA has a role in the repression of germination (Nambara *et al.* 2010). These results suggest that, in barley, *MKK3* is involved in the regulation of grain dormancy through control of the ABA signaling pathway. A splice junction mutant of *MKK3* that can not produce normal *MKK3* protein showed a delayed-germination phenotype (Nakamura *et al.* 2016). Therefore, these results indicate that barley *MKK3* functions as a negative regulator of ABA.

In *MKK3*, the CSP for *Qsd2-AK* is thought to be an adenine (A) residue at the 779th position of exon 7 of the non-dormant KNG allele, which is mutated to a cytosine (C) in the dormant Az allele (Table 1). This nucleotide change causes a non-synonymous amino acid substitution in *MKK3* in the evolutionarily conserved 260th amino acid residue,

changing asparagine (N) to threonine (T). This N260T substitution reduces the kinase activity of *MKK3* (Nakamura *et al.* 2016). Thus, the N260T-substituted dormant *MKK3* allele cannot efficiently transmit phosphorylation signals for germination, delaying germination and conferring the dormant phenotype. These results explain why the dormant *MKK3* allele is recessive (Fig. 2).

MKK3 is expressed in all organs. It participates in the regulation of not only dormancy, but also various abiotic stress response mechanisms such as drought tolerance through the ABA-activated MAPK cascade. Therefore, *MKK3* deficiency should affect not only grain characteristics, but also other phenotypes, such as stomata closure (Zeilcourt *et al.* 2016). This may be why natural null mutation alleles of *MKK3* have not yet been found in barley. The N260T mutation has only a partial effect in decreasing kinase activity and may not be sufficient to severely affect other stress responses or important agricultural traits so that cultivars with the SNP can grow normally in the field.

MFT is the causal gene for the major dormancy QTL *QPhs.ocs-3A.1* in wheat

MFT was identified as the causal gene for the major dormancy QTL *QPhs.ocs-3A.1* in wheat (Nakamura *et al.* 2011). This QTL was detected at the terminal region of the short arm of chromosome 3A using RILs from ‘Zenkouji komugi’ (Zen) and ‘Chinese Spring’ (CS) (Mori *et al.* 2005, Osa *et al.* 2003). Zen was selected from a gamma-ray mutant of Igachikugo-Oregon and released in 1969 (Hoshino *et al.* 2000). Zen is known for its strong dormancy (Miura *et al.* 1997, Osanai and Amano 1993). By contrast, CS is a popular variety widely used for genetic studies worldwide, with far weaker dormancy compared to Zen. CS is thought to have originated from a landrace in the Sichuan region of south China (Sears and Miller 1985). A previous study revealed the existence of genetic similarities between CS and landraces in southwest Japan (Kobayashi *et al.* 2016), indicating that Zen and CS have similar genetic backgrounds.

MFT was first identified in a transcriptome analysis of the effect of temperature on grain dormancy in wheat (Nakamura *et al.* 2011). In barley and wheat, dormancy levels fluctuate depending on the temperature during grain development; for example, lower temperatures during grain development lead to higher dormancy levels (Nakazono *et al.* 2013, Rodríguez *et al.* 2015). Microarray analysis showed that the *MFT* transcript level was higher in embryos that developed at lower temperatures, compared with those that developed at higher temperatures. *MFT* function was confirmed via transient assays, which showed that embryos that expressed *MFT* at high levels (under the control of the maize *ubiquitin* promoter) were unable to germinate. *MFT* was located at the peak of *QPhs.ocs-3A.1*. Furthermore, transgenic complementation analysis showed that introduction of the Zen *MFT* allele into CS led to higher expression of *MFT* and increased dormancy levels of the CS

transformants. Comparison of genomic sequences of Zen and CS identified a SNP responsible for the dormancy attributed to *QPhs.ocs-3A.1* in the *MFT* promoter region.

MFT is a member of the phosphatidylethanolamine binding protein (PEBP) family, which functions in diverse signaling pathways involved in growth and differentiation in bacteria, animals, and plants (Wickland and Hanzawa 2015). In plants, the PEBP family is divided into three sub-families: Flowering locus T (FT), Terminal Flower 1 (TFL1), and *MFT*. All are thought to function as switches for growth phase transitions in the plant life cycle. *FT* and *TFL1* are well-known regulators that determine the timing of flowering; i.e., the phase transition from vegetative growth to reproductive growth. *MFT* is thought to be an ancestral gene for *FT* and *TFL1* because mosses only have an *MFT* homolog. *MFT* functions as a switch for germination, another important phase transition from embryonic growth to post-embryonic growth. *MFT* functions as a suppressor of germination in wheat (Nakamura *et al.* 2011). Subsequent studies suggested that *MFT* has the same function in *Arabidopsis* (Penfield and MacGregor 2017).

The CSP for *QPhs.ocs-3A.1* is a nucleotide substitution from T in CS to C in Zen, 222 bp upstream from the *MFT* initiation codon (−222 SNP) (Table 1). The −222 SNP forms a palindromic sequence (A-motif) in the *MFT* promoter. T in CS is the wild-type conserved allele and C is the mutated allele, which truncates the palindromic sequence. This nucleotide change is presumed to be the cause for the differential *MFT* expression during seed development in Zen. In CS, the expression level of *MFT* is reduced in the final stage of seed development, thus allowing mature grains to germinate. However, in the case of the mutated dormant *MFT* allele, *MFT* expression is not reduced (Nakamura *et al.* 2011). One plausible explanation for this is that some transcription factors, such as bZIP transcription factors, bind the palindromic sequence to stop *MFT* expression at the final stage of grain development. However, the nucleotide change prevents or decreases the binding of these factors and *MFT* continues to be expressed, even during the last, mature stage of seed development. Thus, *MFT* continues to function as a repressor of germination at the mature stage, and in the mature, imbibed grains. Therefore, cultivars with the dormant *MFT* allele have higher levels of seed dormancy.

Although Zen was derived from Igachikugo-Oregon treated with ⁶⁰Co, Igachikugo-Oregon already has the −222 SNP mutation (Nakamura *et al.* 2015). Thus, the mutation did not come from the gamma-ray mutagenesis of Igachikugo-Oregon. The naturally occurring mutation (−222 SNP) in the Zen variety functions as a dominant allele in hexaploid wheat (Fig. 2) and is a very important functional SNP for improving PHS tolerance in wheat. Liu *et al.* (2013) found two mutations at 646 bp and 666 bp (+646 and +666 mutations, respectively) in *MFT*, which they refer to as *TaPHS1*. The +646 and +666 mutations generate a mis-splicing event and a premature stop codon, respectively. Detected using

another parental combination of ‘Rio Blanco’ and ‘NW97S186’, these mutations were found to be CSPs for dormancy in the QTL *Qphs.pseru-3AS* (Liu *et al.* 2013). These two mutations were also found in diploid and tetraploid wheat, indicating that these mutations might have been selected for during domestication of diploid and tetraploid wheat, to decrease dormancy levels of cultivated wheat (Liu *et al.* 2015). These CSPs are loss-of-function mutations of *MFT*, and thus are recessive non-dormant alleles. Although information about these CSPs is useful to exclude them from breeding programs to develop new varieties, they are not useful for conferring higher levels of dormancy. The gain-of-function SNP −222 does increase dormancy levels and is therefore useful for improving PHS tolerance.

MKK3 is also a causal gene for the major dormancy QTL *Phs1* in wheat

A major QTL for dormancy in wheat has been consistently detected on chromosome 4A using various parental combinations (Flintham *et al.* 2002, Kato *et al.* 2001, Mares *et al.* 2005, Torada *et al.* 2005). The same QTL has been given four different names by four different research groups: *Qphs.ocs.1*, *Phs*, *Phs1*, and *Phs-A1*. In this review, the QTL is referred to as *Phs1*, as designated by Torada *et al.* (2016).

During the evolution of hexaploid wheat, a complex chromosomal rearrangement occurred on chromosome 4A (Devos *et al.* 1995). A segment of the terminal region of chromosome 5A translocated to chromosome 4A at the diploid level. Comparative genome analysis indicated that the *Phs1* region in wheat might correspond to the *SD2* locus in barley (Flintham *et al.* 2002, Gao *et al.* 2003, Li *et al.* 2004). Torada *et al.* (2016) recently also identified *MKK3* as the causal gene for *Phs1*, providing direct evidence that *Phs1* in wheat is an ortholog of *SD2* in barley. However, the CSP in *Phs1* is different from that in *Qsd2-AK* in barley (Table 1). For *Phs1*, the CSP is a nucleotide substitution from the wild-type nucleotide C at the 660th position in the dormant allele, to an A in the non-dormant allele. This causes a non-synonymous substitution from the evolutionarily conserved asparagine (N) to lysine (K) in the 220th amino acid residue of the kinase domain. Interestingly, in wheat, the recessive dormant allele has a wild-type, conserved N220, but the dominant, non-dormant allele has a mutated K220 (Fig. 2). This is in contrast to barley, in which the mutant allele is dormant and recessive, and the wild-type allele is non-dormant and incompletely dominant. To date, how the N220K substitution makes the *MKK3* allele dominant and non-dormant remains unknown. This mutation should cause a gain-of-function change in *MKK3*. Since the N220K substitution occurs in the kinase domain, we suspect that the mutation might affect the kinase function of *MKK3*.

East Asia is a nursery for natural mutations that increase PHS tolerance

Mutations in the four known barley and wheat dormancy genes have differing effects on grain dormancy levels. As summarized in Fig. 2, mutations in barley *MKK3* and wheat *MFT* increase dormancy levels, but mutations in barley *AlaAT* and wheat *MKK3* decrease dormancy. The origin of these mutations was deduced from genomic sequence analysis of worldwide collections of wild and cultivated barley and wheat cultivars (Nakamura *et al.* 2015, 2016, Sato *et al.* 2016, Shorinola *et al.* 2017). Interestingly, these geographical studies indicated that the mutations that increase dormancy levels occurred in East Asia, and the mutations that decreased dormancy levels occurred in West Asia.

Analysis of the genomic sequences of wild and cultivated barley identified the direct ancestral allele of the dormant Az *MKK3* allele in barley (Nakamura *et al.* 2016). The ancestral allele has the same genomic sequence as the dormant Az *MKK3* allele, except for the functional SNP that causes the N260T amino acid substitution (Nakamura *et al.* 2016). The ancestral gene has the non-mutated, original nucleotide (A) and therefore, the allele has the evolutionarily conserved amino acid N260. The ancestral allele sequence was found in both wild and cultivated barley. Cultivars collected from Iran, China, and Korea have the ancestral allele sequence, indicating that it traveled from the Fertile Crescent to the northeast of China. Since the dormant *MKK3* allele in barley is mainly distributed in and around East Asia, this indicates that the N260T substitution occurred in East Asia.

Investigation of the geographical distribution of the -222 SNP in the promoter of *MFT* showed that a lot of wheat varieties in Japan have the dormant *MFT* allele, but the non-dormant allele dominates in other regions (Nakamura *et al.* 2015). In this study, only a few varieties from China and Korea were investigated, so the possibility remains that investigation of more wheat varieties from these regions might find more varieties with the dormant *MFT* allele. However, this result suggests that the dormant *MFT* allele is predominantly distributed at least in Japan or perhaps in East Asia.

The study of *MFT* alleles examined approximately 400 European varieties and found only two with the dormant *MFT* allele (Nakamura *et al.* 2015). One of these is 'Ardito', a variety derived from a cross between the line 'Selezione 21' and the Japanese variety 'Akakomugi' by the Italian plant breeder Strampelli in 1913 (Salvi *et al.* 2013). At that time, Akakomugi had two unique, attractive traits for improving lodging tolerance and avoiding drought stress during grain filling: short straw, and extremely early maturation caused by the genes *Rht8* and *Ppd-D1*, respectively. Akakomugi also has the dormant *MFT* allele (Nakamura *et al.* 2015). Thus, we can deduce that the dormant *MFT* allele in Ardito came from Akakomugi, together with *Rht8* and *Ppd-D1*. Lines from this cross soon became well-known in other countries, notably Argentina and China (Salvi *et al.*

2013). This indicates that some of the dormant *MFT* alleles in modern Chinese varieties originated in this lineage.

The cultivation of barley and wheat reached East Asia about four thousand years ago (Fig. 3; Crawford 2006, Feldman 2000, Kidder 1993, Zhang 1983). The climate in East Asia is very different from that of West Asia. The Asian Monsoon, one of the largest-scale seasonal winds in the world, blows from eastern Africa and Madagascar to Japan approximately between May and July (Fig. 3), bringing warm, humid air to East Asia. A lot of rain falls in East Asia around barley and wheat harvest time, which can induce PHS in cultivars with low dormancy, suggesting that when the cultivars reached East Asia, genotypes better adapted to the local climate were selected. Thus, spontaneous mutations for the dormant alleles in wheat *MFT* and barley *MKK3* have been selected and prevailed predominantly in East Asia, and East Asia has acted as a nursery for PHS tolerance genes. Genetic analysis of East Asian cultivars is therefore useful for finding natural mutations to increase dormancy levels for PHS tolerance.

Application of the PHS tolerance genes for breeding programs

Causal genes for the four major dormancy QTL in barley and wheat are now available for marker-assisted selection to improve PHS tolerance in barley and wheat (Table 1).

As Yanagisawa *et al.* (2005) showed in wheat, breeders can develop lines with excellent tolerance to PHS by accumulating natural mutations. However, to develop elite varieties, breeders must also optimize important agricultural traits such as yield, lodging tolerance, and disease resistance. If there is a gene that has deleterious effects on an important agricultural trait closely linked to a dormancy QTL gene, the linkage drag between them could cause a problem for traditional plant breeding approaches. However, in such a case, if we have a marker for the dormancy QTL gene, we can break the linkage using the marker. CSPs themselves might have pleiotropic, deleterious effects on other important agricultural traits. In this case, detailed analysis of the effect of CSPs on gene functions and plant traits will reveal the full effects of the CSPs. This knowledge will inform efforts to utilize the CSPs in breeding programs.

CSP data for the four genes are now ready to use for marker-assisted selection of PHS tolerance genes in breeding programs. Identification of the genes involved in known dormancy QTL provides insight into the uneven distribution of dormancy alleles in cultivars around the world, or even in subregions of the world. For example, wheat varieties in the northern part of Japan do not have the dormant *MFT* allele (Chono *et al.* 2015, Nakamura *et al.* 2015) because they are mainly from the lineage of high quality wheat varieties introduced from North America and Europe (Kobayashi *et al.* 2016). However, transferring the dormant allele from varieties in southern Japan into varieties in northern Japan can improve PHS tolerance in northern Japanese varieties. This

Fig. 3. Migration of barley and wheat cultivars, and a satellite image taken in June in East Asia. BP, before present. The blue arrow shows the direction of winds in the Asian monsoon. The satellite infrared image was taken by the Multifunctional Transport Satellite-2 (MTSAT-2; Himawari-7) in 2014. Green lines show country borders and the world map was obtained from Google Maps.

strategy has already shown promising results.

Outside of Japan, few varieties have the dormant *MFT* allele, so incorporating it into other varieties seems a promising strategy to improve PHS tolerance, in much the same way as *Rht8* from the Japanese wheat variety Akakomugi, or *Rht1* and *Rht2* from Norin10 were introduced into other varieties to develop high yields around the world, before and during the Green Revolution (Hedden 2003, Salvi *et al.* 2013).

The discovery of a recessive dormant allele of the major dormancy QTL *Qsd2-AK* in barley has indicated the possibility of developing novel strategies to improve PHS tolerance in wheat using recessive alleles (Fig. 4). In hexaploid wheat, the N260T mutation is expected to give rise to a strongly dormant allele of *MKK3*, but it has not been selected because the allohexaploid nature of wheat masks the recessive phenotype. Introducing the recessive mutation into all of the functional wheat homoeologs of *MKK3* will be a novel way to increase seed dormancy in wheat.

Another challenge to be solved is how to collect or create specific mutations that are used in breeding programs. It is easier to find knockout mutations of genes than to find specific mutations, but in this case, because *MKK3* is also important in ABA stress signaling pathways (Colcombet *et al.*

Fig. 4. New strategy for improving PHS tolerance of wheat using the N260T mutation in *MKK3*. Allohexaploid wheat has A, B, and D sub-genomes. If we could introduce the N260T mutation into all homoeologous *MKK3* genes, the triple mutant could express its phenotype of increased dormancy, providing a new avenue for developing hyper-dormant wheat cultivars. Red horizontal lines show the locations of the homoeologous *MKK3* genes on each wheat chromosome.

2016), null mutations of *MKK3* carry a high risk of harmful pleiotropic effects on growth or stress tolerance. Developing new ways to efficiently identify specific mutations is the

key to success. Of the four mutations identified, this strategy can be specifically applied to the *Qsd2-AK* mutation because it is the only one known so far to be recessive and to increase dormancy levels.

Conclusions and perspectives

Causal genes for four major grain dormancy QTL in barley and wheat have been identified, increasing our understanding of the regulatory mechanisms of grain dormancy in barley and wheat. From a practical perspective, as a recent paper showed that the *MFT* dormant allele can be used to improve PHS tolerance in durum wheat (*Triticum turgidum* ssp. *durum* (Desf.) Husn.) (Kato *et al.* 2017), identification of these genes paves the way for marker-assisted selection to confer PHS tolerance, and for increasing dormancy levels of hexaploid wheat using the recessive *MKK3* dormant allele. These results have informed strategies for breeding of grain dormancy or PHS tolerance in barley and wheat. However, this is just the beginning of the study on natural mutations in grain dormancy in barley and wheat. There are other known dormancy QTL in barley and wheat (Cao *et al.* 2016, Gong *et al.* 2014, Hickey *et al.* 2012, Nakamura *et al.* 2017, Somyong *et al.* 2014) for which the causal genes remain to be identified. Future QTL studies in model plants such as *Arabidopsis* and rice will reveal more causal genes (Bentsink *et al.* 2010, Marzougui *et al.* 2012), and with the rapid progress of studies in seed dormancy and germination (Nonogaki 2017), findings will no doubt facilitate the identification of other causal genes for dormancy QTL in barley and wheat, and eventually, breeding of PHS tolerant barley and wheat cultivars will be realized.

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